High temperatures reveal cryptic genetic variation in a polymorphic female sperm storage organ

Berger, David ; Bauerfeind, Stephanie S ; Blanckenhorn, Wolf U ; Schäfer, Martin A

Abstract: Variation in female reproductive morphology may play a decisive role in reproductive isolation by affecting the relative fertilization success of alternative male phenotypes. Yet, knowledge of how environmental variation may influence the development of the female reproductive tract and thus alter the arena of postcopulatory sexual selection is limited. Yellow dung fly females possess either three or four sperm storage compartments, a polymorphism with documented influence on sperm precedence. We performed a quantitative genetics study including 12 populations reared at three developmental temperatures complemented by extensive field data to show that warm developmental temperatures increase the frequency of females with four compartments, revealing striking hidden genetic variation for the polymorphism. Systematic genetic differentiation in growth rate and spermathecal number along latitude, and phenotypic covariance between the traits across temperature treatments suggest that the genetic architecture underlying the polymorphism is shaped by selection on metabolic rate. Our findings illustrate how temperature can modulate the preconditions for sexual selection by differentially exposing novel variation in reproductive morphology. This implies that environmental change may substantially alter the dynamics of sexual selection. We further discuss how temperature-dependent developmental plasticity may have contributed to observed rapid evolutionary transitions in spermathecal morphology.

DOI: https://doi.org/10.1111/j.1558-5646.2011.01392.x

High temperatures reveal cryptic genetic variation in a polymorphic female sperm storage organ

D. Berger[1], S.S. Bauerfeind[1,2], W.U. Blanckenhorn[1] and M.A. Schäfer[1]

[1] Institute for Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[2] Zoological Institute and Museum, University of Greifswald, Johann-Sebastian-Bach Str. 11/12, 17489 Greifswald, Germany

Corresponding author:
David Berger
Institute for Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
Tel.: +41 (0)44 635 47 73
Fax: +41 (0)44 635 47 80
Email: david.berger@ieu.uzh.ch

Running title: Evolvability of female reproductive morphology

Key words: sexual selection, developmental plasticity, canalization, reproductive morphology, population genetics, quantitative genetics.

Abstract

Variation in female reproductive morphology may play a decisive role in reproductive isolation by affecting the relative fertilization success of alternative male phenotypes. Yet, knowledge of how environmental variation may influence the development of the female reproductive tract and thus alter the arena of post-copulatory sexual selection is limited. Yellow dung fly females possess either three or four sperm storage compartments, a polymorphism with documented influence on sperm precedence. We performed a quantitative genetics study including 12 populations reared at three developmental temperatures complemented by extensive field data to show that warm developmental temperatures increase the frequency of females with four compartments, revealing striking hidden genetic variation for the polymorphism. Systematic genetic differentiation in growth rate and spermathecal number along latitude, and phenotypic covariance between the traits across temperature treatments suggest that the genetic architecture underlying the polymorphism is shaped by selection on metabolic rate. Our findings illustrate how temperature can modulate the preconditions for sexual selection by differentially exposing novel variation in reproductive morphology. This implies that environmental change may substantially alter the dynamics of sexual selection. We further discuss how temperature-dependent developmental plasticity may have contributed to observed rapid evolutionary transitions in spermathecal morphology.

Introduction

Comparative analyses of female reproductive organs show a pattern of rapid evolutionary transition (Pitnick et al. 1999, Jennions and Petrie 2000, Swanson and Vacquier 2002, Beese et al. 2008, Prokupek et al. 2010, Puniamoorthy et al 2010) and recent theoretical and empirical research has further identified post-copulatory sexual selection as an important driving force in generating this variation (Eberhard 1996, Hosken and Stockley 2004, Arnqvist and Rowe 2005, Pitnick et al. 2009, Birkhead 2010). Complementary lines of research comprising comparative studies (e.g. Presgraves 1999, Beese et al. 2006, Rönn et al. 2007), experimental evolution (e.g. Hosken et al. 2001) and sperm competition experiments (e.g. Ward et al. 2000, Miller and Pitnick 2002) also suggest that the female reproductive tract functions as the arena for sperm choice and sexual conflict. Nevertheless, studies failing to link variation in female reproductive morphology to sexual selection are numerous (Ritchie 2007, Kraaijeveld et al. 2010, Snook et al. 2010). Logically therefore, traditional explanations for divergence in genitalia invoking neutral mechanisms of evolution such as genetic drift and pleiotropy (e.g. Dobzhansky and Holz 1943, Mayr 1963) have received renewed interest (Arnqvist 1997, Civetta and Singh 1998, Ellegren and Parsch 2007, Van Dyken and Wade 2010).

The role of environmental perturbations in generating rapid macroevolutionary transitions in morphological traits by exposing hidden variation to selection has recently been reemphasised (Debat and David 2001, West-Eberhard 2003, Schlichting 2008, Lande 2009). A trait's phenotypic expression and functional relationships with other traits are predicted to depend on the environmental settings (Houle 1991, Lynch and Walsh 1998, McGuigan and Sgro 2009) and in many cases phenotypic plasticity has evolved as an adaptive response for optimizing performance given a set of conditions (Schmalhausen 1949, Stearns 1989). Stressful or rare environmental

conditions may on the other hand expose novel phenotypes through effects on developmental and metabolic pathways because selection for developmental buffering against the environmental perturbation has been absent in the past (Schlichting 2008, McGuigan and Sgro 2009). In some such cases extreme environments may reveal alternative developmental pathways previously under selection for refined functioning but currently hidden away from selection due to contemporary conditions (West-Eberhard 2003, Moczek 2009).

Environmental effects on the development of reproductive organs may be expected to be modest assuming that the reproductive tract is under strong stabilizing selection for canalized development due to its involvement in fertilization. On the other hand, traits with sex-specific expression are expected to accumulate more genetic variance under mutation-selection balance because they are exposed to selection only half as often as traits expressed in both sexes (Ellegren and Parsch 2007, Van Dyken and Wade 2010). Reproductive organs are consequently expected to show a relative lack of developmental canalization (Civetta and Singh 1998, De Visser et al. 2003). While investigation of genotype by environment interactions has been carried out in some studies on male genitalia (Arnqvist and Thornhill 1998, Andrade et al. 2005, Soto et al. 2008, Shingleton et al. 2009) comparatively little appreciation has been given to the importance of environmental variation for the development of female reproductive organs. This is unfortunate because environmentally induced morphological variation in the female reproductive tract, even when selectively neutral *per se*, changes the physical conditions at the site of fertilization and may affect the relative success of alternative male phenotypes (Clark et al. 1999, Tregenza et al. 2009, Pitnick et al. 2009). Environmental heterogeneity may thus induce spatio-temporal differences in the conditions of post-mating sexual selection.

Arthropods are perhaps the group of animals for which reproductive morphology has been studied in most detail. With an ectothermic physiology, arthropods are directly dependent on temperature and have therefore also been subject to detailed study with respect to current and future impacts of climate change. While effects of temperature have been reported to induce phenotypic change in most if not all developmental processes and life history traits investigated to date (Hochachka and Somero 2002, Angilletta 2009), less is known about how temperature variation affects reproductive morphology. This may seem paradoxical in light of the direct relationship between fertilization success and population fitness. In a more positive and intriguing sense, temperature-induced differences in reproductive morphology may promote local adaptation to changing environments by facilitating assortative mating among ecologically segregating demes (Kondrashov and Kondrashov 1999, Ritchie 2007). We here investigated the putative role of temperature in shaping and maintaining large-scale geographic variation in the polymorphic female sperm storage organ of the yellow dung fly, *Scathophaga stercoraria*, arguably one of the best studied systems for postcopulatory sexual selection (e.g. Parker and Simmons 1994, Otronen et al. 1997, Simmons et al. 1999, Ward 2000, Hosken et al. 2001, Sbilordo et al. 2009, Bussiere et al. 2010, Thüler et al. 2011).

Spermathecae are sclerotized compartments used by female arthropods to store sperm over extended periods of time. Spermathecal morphology varies greatly among even closely related taxa and has also been shown to covary with male reproductive structures (e.g. Dybas and Dybas 1981, Pitnick et al. 1999, Presgraves et al. 1999, Rivera et al. 2004, Minder et al. 2005, Beese et al. 2006; 2008, Sauer and Hausdorf 2009). Variation in the number of spermathecal compartments has received considerable interest because theoretical models have shown that multiple storage sites might facilitate cryptic sperm choice if females are able to store and utilize

sperm from different males non-randomly (Hellriegel and Ward 1998). This premise has repeatedly been demonstrated in the yellow dung fly (e.g. Otronen et al. 1997, Simmons et al. 1999, Ward 2000). The sperm storage organ of the yellow dung fly typically consists of three spermathecae (3s-phenotype), but some females develop four compartments (4s-phenotype) (see fig 1). Intermediate phenotypes with bifurcated spermathecae also exist illustrating the developmental transition between alternative phenotypes (Ward et al. 2008). The species thus presents a unique opportunity to study the putative functional and developmental mechanisms responsible for the major phylogenetic transitions in spermathecal numbers apparent within multiple independent arthropod clades. By performing sperm competition experiments using artificial selection lines, Ward (2000) found that females with four spermathecae show reduced last male sperm precedence, indicative of cryptic female choice. Because selection for the additional spermatheca also lead to a small reduction in female fecundity it has been hypothesized that a balance between genetic benefits through female choice and life-history costs maintains the polymorphism within natural populations (Ward 2008). However, little is known about natural variation in the trait.

Previous studies on natural populations of the yellow dung fly using common garden designs have reported local adaptation in response to both sexual selection (Hosken et al. 2002, Kraushaar and Blanckenhorn 2002) and to climate and seasonal time constraints (Demont et al 2008, Scharf et al 2010). We here employed a similar population genetics approach which included rearing of 12 European yellow dung fly populations (fig 2) at three contrasting developmental temperatures. This allowed us to perform a quantitative genetics analysis to i) look for evidence of selection maintaining large scale geographical variation in spermathecal number, and ii) investigate the role of temperature in shaping phenotypic and genetic variance among the studied populations.

To look for selection we performed direct comparisons of standardized measures of population divergence (Qst) in spermathecal number with simultaneously scored Qst for life history traits and previous estimates of neutral genetic divergence (Fst) across Europe (Demont et al. 2008). We further investigated the temperature-specific genetic architecture underlying the spermatheca dimorphism and tested for latitudinal clines in three life history traits (body size, development time, growth rate) and spermathecal number. We also investigated putative relationships between spermathecal number and life history variation by calculating phenotypic and genetic correlations between the traits within the respective developmental temperature treatments. We finally dissected a large number of wild caught flies to look for spatio-temporal patterns in the 4s-expression in the wild and to relate this natural variation to the temperature-specific patterns of phenotypic and genetic variance in the spermatheca polymorphism revealed by the laboratory rearing.

Methods

Common garden design and wild sampling

We raised 12 populations sampled over a broad latitudinal range in a common garden. In the spring of 2007 five populations, in 2009 seven populations, were collected by capturing mating pairs from dung pats at cow pastures (fig 2, table A1 in supl. material). The inclusion of a large number of full-sib families per population allowed us to perform an extensive quantitative genetics survey of population differentiation and the distribution of phenotypic and genetic variances in spermathecal number across Europe. We followed the general procedures for

166 capturing, rearing and maintaining yellow dung flies as described in Blanckenhorn et al. (2010).
167 Here we briefly describe the specifics of our common garden design.
168
169 In both years, females were brought to the laboratory where they were allowed to lay eggs on
170 cow dung that had been deep-frozen (-80°C) before use in order to exclude inadvertent
171 competitors. All F1-offspring were reared through at 18°C. At adult eclosion, males and females
172 from different F1-familes were randomly mated to form the new F2-generation consisting of the
173 female offspring examined in this study. The use of F2-progeny minimizes differences among
174 populations due to possible maternal effects. We allowed only unique F1-combinations of males
175 and females to mate and form the F2-generation yielding 374 families split among the 12
176 populations (table A1). Eggs from the F1 mothers were split in groups of 15 among three 30 ml
177 rearing bottles containing ad libitum dung for larval feeding (30 grams, see Blanckenhorn et al
178 (2010)). The rearing containers were then randomly assigned to either 12°C, 18°C or 24°C with a
179 14:10h L:D cycle. These temperatures represent natural growing conditions (12°C-18°C) up to
180 very warm conditions (24°C) that may induce adult flies to aestivate during summer periods
181 (Blankenhorn et al. 2010), thus enabling observation over a broad range of environmental values.
182
183 Once per day rearing bottles were checked for emerging flies to score development times. The
184 flies were frozen upon adult emergence to be later dissected for spermathecal number and
185 measured for hind tibia length as an estimate of body size. Dividing tibia length by development
186 time produced a linearized estimate of growth rate. Some of the 12°C flies entered diapause
187 which is obvious from the bimodal distribution of development times at this temperature. We
188 waited for all flies to emerge and also counted the spermathecae and measured body sizes of
189 diapausing females, though we did not calculate growth rates or score development times for

these individuals (development time > 70 days). Spermathecal phenotypes did not differ between diapausing and directly developing females so we included all flies in the analysis of spermathecal number.

Male reproductive traits are predicted to coevolve with female reproductive morphology and intersexual correlations may also arise between analogous male and female structures due to a shared developmental basis. However, quantifying variation in male reproductive traits for a study of this size was not feasible. Thus, we here focus only on variation in females. For a survey of intersexual correlations in reproductive morphology (excluding spermathecal number) in the yellow dung fly we refer to Thüler et al. (2011). In total we analyzed 4825 females for the spermatheca dimorphism. As fewer females were measured for body size, analyses of the four traits were based on different numbers of females (table 1).

We finally estimated the spatial and temporal distribution of spermatheca phenotypes in nature by dissecting 405 wild caught females used to establish our common garden populations plus an additional 529 flies of the northern Swiss population collected over the entire flight season (table A1).

We analyzed the common garden data primarily by performing generalized mixed linear models using the lme4-package available for R (Statistical and Computation Team 2005), with full-sib families as random effects and population and temperature as fixed effects. We calculated p-values by Wald-statistics using likelihood-ratio tests comparing models with and without the specific effect of interest. The effect of year was always blocked by adding it as a factor in all analyses.

214

215 ***Bayesian approximations of variance components and Qst:s***

216 We compared population differentiation and underlying genetic variance in the three rearing
217 temperatures by calculating the amount of variation explained by populations and families within
218 populations. We further calculated standardized measures of genetic differentiation in the
219 spermatheca dimorphism (Qst) to compare it to corresponding estimates for the three life history
220 traits and to previously published data on neutral genetic differentiation (Fst) in this species
221 across Europe (Demont et al. 2008).

222

223 Assuming that the threshold expression of a binomial trait (here spermathecal number) has an
224 underlying normally distributed genetic value this latent variable can be estimated as a liability
225 trait by using link-functions in generalized linear models (Lynch and Walsh 1998, Goldstein et al
226 2002). Estimating variance components for binomial traits is a demanding task, especially with
227 unbalanced designs, and standard maximum likelihood procedures may work poorly in such cases
228 (Bolker et al 2008, Nakagawa and Schielzeth 2010). We therefore complemented restricted
229 maximum likelihood (REML) estimates provided by the mixed models with Bayesian analyses.
230 The "MCMCglmm"-package for R uses additive overdispersion models with a logit-link function
231 and Monte Carlo simulation. These analyses allow assessment of variance components and their
232 95% credible intervals (the non-parametric Bayesian equivalent of confidence intervals) by
233 estimation of overdispersion in the data. This overdispersion can then be assigned to hierarchical
234 nesting structures such as family- or population level variance (Hadfield 2010). One inherent
235 problem of analysis is that the residual variance cannot be calculated but must be set to a fixed
236 value (Goldstein et al. 2002). Consequently, all other variance components scale relative to the
237 residual variance. However, the intraclass correlation denoting the relative amount of variation

explained by the desired factor remains unaffected by this arbitrary scaling and thus can be estimated. In all analyses we blocked the effect of year. To allow Bayesian estimation of population and family variance components these were incorporated as random effects with standard weak priors as recommended by Hadfield (2010). Changing priors within reasonable limits did not change results. Our approximations of intraclass correlations (IC) denoting the relative family- and population effects at each temperature are given by:

$$IC_{fam} = V_{fam} / (V_{fam} + V_{res} + \pi^2/3)$$

$$IC_{pop} = V_{pop} / (V_{pop} + V_{fam} + V_{res} + \pi^2/3)$$

where $\pi^2/3$ is equal to the variance for the standard logistic distribution (Goldstein et al 2002) that was added when calculating the components for spermathecal number. Note that since we used a full-sib design the intraclass correlation for the family effect should be doubled to give the broad sense heritability. Environmental differences between rearing containers together with random maternal effects may contribute to differences between families leading to overestimation of heritabilities. However, our main objective was not to infer a quantitative measure of heritability, but to compare the genetic components of the different traits across the three temperatures. We therefore settled on contrasting the raw family intraclass correlations for this relative and unbiased comparison.

We can further produce estimates of Qst (reformulated from Spitze (1993)):

$$Qst = V_{pop} / (V_{pop} + 2V_{fam} / f)$$

where *f* refers to the coefficient of relatedness between subjects within families which in our case is equal to 0.5. Our estimates of family variance (V_{fam}) are based on total and not additive genetic variance and as mentioned above may include maternal- and other common environment effects, thus our Qst-values are likely to be conservative estimates (Whitlock 2008). For an extended discussion on the interpretation of these variance components see table 1.

Results

Thermal plasticity and genetic variance in the spermatheca polymorphism

To investigate temperature-dependent developmental plasticity in spermathecal number we first performed one analysis on the whole dataset including all three temperature treatments. We then continued by analyzing each treatment separately to estimate temperature-specific population and family effects (for population effects see below). As revealed by the analysis on the whole dataset, spermathecal number showed striking developmental plasticity with the frequency of the 4s-phenotype strongly increasing with temperature (Chi-2 = 305.8, df = 16, $p < 0.001$) (fig 3). There was no significant interaction between population and temperature on spermathecal number (Chi-2 = 22.6, df = 38, $p = 0.42$) indicating that spermathecal number responded similarly to temperature across populations. There was also a strong main effect of family (Chi-2 = 90.2, df = 16, $p < 0.001$). Conducting analyses for each treatment separately showed that the family effect strongly increased with developmental temperature (table 1) indicating not only greater 4s-expression and phenotypic variance, but also more genetic variance for the dimorphism at warm temperatures. The corresponding Bayesian analyses supported these results showing higher intraclass correlations for the family effects at warmer temperatures (table 1). The

phenotypic variance (V_P) of a binomial trait is equal to the product of the frequencies of the two outcomes: p*(1-p). The broad sense heritability (h^2) can further be approximated as twice the intraclass correlation for the family effect with our full-sib design. We can thus estimate the total genetic variance (V_G) for the spermatheca dimorphism as V_P*h^2. This exercise confirmed that there is more measurable (i.e. exposed) genetic variance for the polymorphism at warm temperatures (fig. 4).

As expected, the corresponding REML and Bayesian analyses for the three life history traits all showed highly significant effects of temperature (for growth rate see fig 3) and family nested within populations. Notably and contrary to the results for spermathecal number, there was abundant genetic variance for all traits regardless of temperature indicated by strong family effects in the REML analyses and high intraclass correlations in the Bayesian analyses (see details in table 1).

Population differentiation and Qst-estimates

The analysis including all three temperature treatments showed an overall weak effect of population on spermathecal number (Chi-2 = 22.6, df= 16, p = 0.013) providing evidence for geographical differentiation in the polymorphism. Still, calculations of population differentiation and Qst:s for spermathecal number conducted for each of the three developmental temperature separately estimated Qst:s close to zero, implying that spermathecal number evolves neutrally. However, the very low 4s-expression at the 12°C treatment did not supply enough statistical power to properly estimate the Qst for this binomial trait at this setting (table 1) (see also below). In contrast to the low Qst:s for spermathecal number in 18°C and 24°C, the majority of the lower credible limits for life history Qst:s were non-overlapping with the previous estimate of neutral

genetic differentiation among 10 European populations (Fst = 0.007) (Demont et al. 2008 cf. fig 1) implying local adaptation in these traits (table 1).

Latitudinal clines in spermathecal number and life history traits

As the formation of the spermathecae showed strong temperature-dependence and latitudinal patterns of thermal adaptation in life history have been demonstrated previously in this species (Demont et el. 2008, Scharf et al. 2010), we continued by testing for latitudinal population clines in both life history and spermathecal number at the three temperatures using standard ANCOVAS. For all traits we extracted population means (for the spermatheca dimorphism expressed as each population's 4s- frequency) before regressing them on population latitude. As in previous analyses the year effect was blocked. The analysis also provided an alternative to the variance partitioning methods used above which suffered from low statistical power for the analysis of spermathecal number at 12°C.

We found a latitudinal cline in spermathecal number at 12°C with northern populations exhibiting higher 4s-phenotype frequencies than southern populations ($F_{1:9} = 8.90$, $p = 0.015$, $R^2 = 0.50$) (fig 5). This effect was not apparent at 18°C ($F_{1:9} = 0.71$, $p = 0.42$), nor at 24°C ($F_{1:9} = 0.81$, $p = 0.39$). Strikingly, latitudinal patterns were similar for the analyses on growth rate with northern populations growing significantly faster than southern populations at 12°C (growth rate: $F_{1:9} = 6.97$, $p = 0.027$, $R^2 = 0.44$) (fig 5) and similarly, no clines at 18°C ($F_{1:9} = 0.31$, $p = 0.59$) or 24°C ($F_{1:9} = 0.12$, $p = 0.74$). The same pattern emerged when analyzing development time with northern populations developing faster at 12°C ($F_{1:9} = 5.53$, $p = 0.043$) and no differences at the other temperatures (18°C: $F_{1:9} = 0.99$, $p = 0.35$, 24°C: $F_{1:9} = 0.28$, $p = 0.61$). No significant clines

were found for body size (12°C: $F_{1:9} = 3.02$, $p = 0.12$, 18°C: $F_{1:9} = 0.03$, $p = 0.87$, 24°C: $F_{1:9} = 0.00$, $p = 1$).

Phenotypic- and genetic correlations between spermathecal number and growth rate

The parallel latitudinal clines in growth rate, development time and spermathecal number as well as their similar pattern of thermal plasticity suggest a direct linkage and a positive relationship between the rate of development and the expression of an additional sperm storage compartment. We therefore continued by testing for correlations between growth rate and spermathecal number within the temperature treatments. We controlled for population effects in standard ANOVAs with growth rate as the response variable and spermatheca phenotype as a two-level factor. There was no difference between 3s and 4s females at either 12°C ($F_{1:492} = 0.22$, $p = 0.64$) or 18°C ($F_{1:1157} = 0.38$, $p = 0.54$), whereas growth rates of 4s females were marginally faster at 24°C ($F_{1:986} = 4.30$, $p = 0.038$, growth rate 4s = 0.559, 3s = 0.551). In an attempt to minimize effects of variation in individual rearing conditions or in genetic quality on trait covariance, we also tested if sibling offspring reared together within the same bottle differed in their growth rate depending on their spermatheca phenotype by nesting females within rearing bottles in a mixed model. We found no significant relationship between spermatheca phenotype and growth rate, although 4s-phenotypes tended to grow faster overall (Chi-2 = 2.89, df = 6, $p = 0.089$). An analysis based on population means showed that populations that on average grow fast also tended to have a higher expression of the 4s-phenotype indicating a positive genetic correlation, although the effect was not statistically significant (Chi-2 = 2.99, df = 7, $p = 0.084$). We also performed analyses with development time as the response variable that gave similar results.

We estimated genetic correlations between growth rate and spermathecal number by calculating both Pearson and Spearman's rank (because residuals were not normally distributed) family mean genetic correlations for the traits across the three developmental temperatures. These two analyses provided almost identical results so we here report only on the results from the Pearson correlations. We only included families with complete observations for all traits across all treatments assuring that families were well replicated. This resulted in 145 families for analysis. Population effects were blocked. This analysis showed a significant positive genetic correlation for 4s-phenotype frequency between 18°C and 24°C ($r = 0.31$, $p < 0.001$) which is in agreement with previous findings suggesting a high heritability of this trait in laboratory settings (Ward 2000, Ward et al 2008), but no correlations with expression at 12°C ($p > 0.20$). Growth rate showed a similar pattern with the highest correlation between growth at 18°C and 24°C ($r = 0.30$, $p < 0.001$) but also a significant relationship between growth at 12°C and 18°C ($r = 0.26$, $p = 0.002$). However, there were no significant correlations between growth rate and 4s-expression at any temperature ($r = 0.04$-0.09, $p > 0.28$). Thus, even though the two traits have seemingly similar underlying physiological sensitivity to temperature and show similar patterns of latitudinal variation, there seems to be little genetic variation within populations at pleiotropic loci.

Natural variation in spermathecal number

Dissections of the large sample of wild caught flies across latitude and season showed that the overall frequency of the 4s-phenotype in nature is very low (1.4%, fig 3) which could be expected given that the 12°C laboratory environment, resembling natural temperature conditions, also showed low 4s-expression. Of in total 934 caught and dissected females from the wild, only 13 showed the 4s-phenotype and these were equally distributed across latitude and season. Our

sample size is large and covers 529 Swiss females caught from spring to autumn and an additional 405 females sampled between Norway and Spain (table A1). Thus, any effects of natural temperature variation on the expression of the spermatheca polymorphism must be moderate and we conclude that most of the observed phenotypic variation in this trait among adult females remains hidden from selection in the wild.

Discussion

The spermathecal polymorphism in the yellow dung fly has previously been hypothesized to be maintained through its possible role in mate choice (Ward 2000, Ward et al. 2008). Here we demonstrate that the expression of the alternative spermatheca phenotypes is strongly temperature-dependent. This is also reflected by clinal variation in spermathecal number across a broad latitudinal range. Dissections of wild caught females further show that the alternative 4s-phenotype is extremely rare in the wild, indicating that the polymorphism is hidden from direct selection. In the following we first discuss the importance of temperature-dependent developmental plasticity of female reproductive morphology in driving antagonistic male-female coevolution and geographic differentiation. We then turn focus to the putative role of temperature perturbations in inducing developmental plasticity and rapid evolutionary transitions in reproductive morphology by exposing novel phenotypes to selection.

Thermal plasticity, pleiotropy, and geographic variation in reproductive morphology

We found parallel clines in 4s-frequency, development time and growth rate at cold temperature, and similar plastic responses of all three traits to temperature. This could imply that the clinal pattern in spermathecal number in the yellow dung fly is driven by pleiotropic effects of genes

404 experiencing latitude-specific selection on physiology. Genetic correlations calculated within
405 temperature treatments and populations were all positive but give little support for strong direct
406 genetic linkage between growth rate and the 4s-expression. However, given that the traits are
407 very different and any pleiotropic loci likely reside upstream in the gene network (e.g. governing
408 a shared metabolism), the genetic correlation between the two traits is expected to be weak and
409 thus may not easily be verified statistically (Lynch and Walsh 1998). High autonomy (and thus
410 weak pleiotropy) is further expected for cryptic variation, which, due to its hidden nature
411 nevertheless may readily evolve through pleiotropic selection (McGuigan and Sgro 2009). The
412 parallel phenotypic responses of growth rate and 4s-expression in this study have been replicated
413 with additional treatments manipulating growth rate through dung dilution or adding of chemical
414 toxins (MA Schaefer, L Bussiere, Berger D and WU Blanckenhorn, unpublished data). This
415 supports the hypothesis that these traits are regulated by common underlying metabolic pathways.
416 Local adaptation in growth and development along latitude in response to climate has been
417 demonstrated for the yellow dung fly (here and previously: summarized in Blanckenhorn 2009).
418 Selection on faster metabolism at high latitudes therefore seems to be one likely explanation for
419 the clinal variation in spermathecal number found here. The observation of clinal variation in life
420 history and spermathecal number only in the 12°C treatment further indicates that phenotypic
421 effects of variation at loci under selection are specific to cold environments.
422
423 Regional difference in environmental factors affecting reproductive organs, either directly or
424 through effects on correlated traits may be a potent agent generating rapid geographical
425 differentiation. Although more often stressed in studies investigating male genitalia, a few other
426 studies have found significant correlations between female sperm storage organ morphology and
427 ecological factors or life history variation (Miller and Pitnick 2003, Amitin and Pitnick 2007,

Beese et al. 2008, Phiancharoen et al. 2010). Such environmentally induced differences in reproductive morphology represent a mechanism that can mediate assortative mating among geographically diverging populations and thus may be a persuasive force contributing to reproductive isolation and local adaptation (Kondrashov and Kondrashov 1999, Ritchie 2007, Kraaijeveld et al. 2010).

The implications of environmental regulation of reproductive morphology for processes of population divergence will depend on the degree and nature of phenotypic plasticity. If morphology is under strong stabilizing selection, geographical populations may for example be expected to evolve different levels of developmental buffering against environmental variation which seems a likely explanation for the population differences in spermathecal number reported here for the yellow dung fly. In some cases, male and female counterparts may respond very differently to environmental perturbation generating population-specific coevolution driven by the particular environmental settings at the geographical region. Such differential plasticity is likely if male and female morphology has fundamentally different genetic backgrounds and originate from different developmental tissues (De Visser et al. 2003, West-Eberhard 2003). On the other hand, the effects of temperature as studied here typically induce general responses in ectotherm physiology. For example, all metabolic processes typically speed up in warm temperatures (Hochachka and Somero 2002). Warm developmental conditions are also known to reduce organism body size over most of the biologically relevant temperature range (*The Temperature-Size Rule*, Atkinson 1994). This pattern also applies for organ and cell size in general (Angilletta 2009). Therefore, changes in some environmental variables such as temperature are perhaps more likely than others to generate geographic covariation in sexually antagonistic traits such as the size of male testes and female sperm storage organs.

Developmental plasticity in spermathecal number

Previous studies have suggested that the spermatheca polymorphism in the yellow dung fly is maintained within natural populations by a balance between genetic benefits through female choice and energetic costs associated with the development of an additional spermatheca (Ward et al. 2008). We found no evidence for energetic trade-offs associated with spermathecal development in terms of negative correlations with growth rate, rate of development or body size. In addition, attempts to repeat the result from Ward et al. (2008) finding weak fecundity costs associated with the 4s-phenotype are equivocal (M.A. Schäfer, L. Bussiere, D. Berger and W.U. Blanckenhorn, *unpublished data*). Our data instead show that the frequency of females carrying four spermathecae consistently increase with developmental temperatures even when they are detrimentally high (24°C reduces fitness, see Teuschl et al. 2007). The phenotypic robustness of spermathecal number in the wild therefore seems likely to be the result of canalization of spermathecal formation and development in general. Conversely it seems unlikely that the substantial phenotypic and genetic variance expressed at warm temperatures reflect adaptive phenotypic plasticity but more likely trait decanalization explained by conditionally expressed loci with no noticeable or even beneficial effects under natural conditions. In addition, if under strong selection we would expect the dimorphism to behave like a threshold trait with precise environmental regulation (West-Eberhard 2003, Moczek 2009). The occurrence of the 4s-phenotype throughout the whole temperature-gradient studied instead implies that the genetic control mechanism governing the temperature-sensitivity of spermathecal number is under relaxed selection. This premise is further supported by our Qst-estimates, which at warm temperatures did not differ from neutral expectations. This contrasts with the significant genetic

476 differentiation found for the studied life history traits illustrating that possible swamping effects
477 of gene flow are no insurmountable obstacle to local adaptation among these populations (see
478 also Demont et al. 2008, Scharf et al. 2010).
479
480 Population genetics theory predicts low genetic robustness in reproductive morphology under
481 mutation-selection balance as a result of their sex-specific expression (De Visser et al. 2003, Van
482 Dyken and Wade 2010). Previous studies investigating the developmental and genetic basis of
483 spermathecal morphology have also found similar patterns of developmental plasticity as in our
484 study. Wexelsen (1928) found that the development of spermathecaea in *Drosophila* showed
485 temperature sensitivity. Similarly, Mather and Harrison (1949) used *D. melanogaster* to
486 demonstrate increases in spermathecal number both in response to high developmental
487 temperature and to correlative selection on chaeta number. More recently Allen and Spradling
488 (2008) showed that different mutations in the nuclear hormone receptor gene *Hr39* generates
489 alternative phenotypes with numbers of spermathecae varying from none up to three in
490 comparison to the typical *Drosophila* wild-type carrying two spermathecae. Another remarkable
491 example was presented by Kamimura (2007) who showed that the typically single unbranched
492 spermatheca in two species of earwigs exhibits particular sensitivity to radiation; with increased
493 dosage the single spermatheca branched into seven. We have similarly recorded a few individuals
494 with 5, 6 and 8 spermathecae respectively when raising yellow dung flies under warm laboratory
495 conditions. Immunohistochemical data on the roundworm *Caenorhabditis elegans* show that heat
496 shock proteins (Hsp) are overrepresented and over-expressed in the spermathecal tissue during
497 development (Ding and Candido 2000). The role of Hsp in capacitating phenotypic diversity
498 through buffering against environmental stress has been demonstrated in diverse taxa such as
499 insects, flowering plants, and yeast (reviewed in: Sangster et al. 2004). Artificial reduction of

Hsp-function transforms the link between genotype and phenotype and typically exposes a range of novel morphological varieties. This response also mirrors and exaggerates the response induced by temperature stress alone (Rutherford and Lindquist 1998). The study by Ding and Candido (2000) may thus hint at a molecular mechanism behind the observed temperature-sensitivity of spermathecal development.

Hidden genetic variation and rapid evolutionary transitions

Waddington's (1953) classical demonstration of temperature-dependent genetic assimilation in *D. melanogaster* highlighted the importance of cryptic genetic variation for major evolutionary transitions. Modern insights into the molecular and developmental mechanisms governing phenotypic diversity have put new emphasis on the importance of such variation (e.g. West-Eberhard 2003, Schlichting 2008, Lande 2009, McGuigan and Sgro 2009). Recent examples of dynamic reversible evolution through gain and losses of complex morphologies include wing-spots (Prud'homme et al. 2006) and sex combs (Tanaka et al. 2009) in *Drosophila*, and the evolution of horned male morphs in dung beetles (Moczek 2009). Suzuki and Nijhout (2006) provided a particularly enlightening example illustrating how genetic assimilation and accommodation of novel variation exposed by temperature perturbation may have contributed to the diversification in larval colour dimorphism in *Manduca* moths. The evolution of spermathecal number across the *Diptera* shows a similar pattern of reversible evolution. Interestingly, the rare occurrence of the 4s-phenotype in the wild reported here suggests that fixation of 4s- and 3s-phenotypes in previous artificial selection experiments on yellow dung fly females (Ward 2000, Thüler 2009) was accomplished through genetic assimilation and accommodation respectively. It was further found that last male sperm precedence was significantly lower in females with four spermathecae (Ward 2000). This could be interpreted as that female sperm sorting had been

affected by the genetic recruitment of an additional storage compartment, thus demonstrating a potential function of the novel phenotype. We have further demonstrated variation in spermathecal number in *Scathophaga suilla,* a close relative occurring sympatrically with *S. stercoraria*. In *S. suilla* however, the 4s-phenotype is much rarer and also shows less sensitivity to temperature (MA. Schaefer and D. Berger, unpublished), indicating that the underlying developmental mechanism has evolved differences in the two species. The dipteran phylogeny exemplifies various transitions in spermathecal number across taxa with most species in the suborder *Brachycera* having 1-3 spermathecae where 3 appears to be the ancestral state (Yeates 2002). Additional recordings of four spermathecae have also been made for some additional taxa (*Dolichopodidae*, D. Berger, unpublished data). More detailed studies in *Drosophila* (Pitnick et al. 1999), stalk-eyed flies (Presgraves et al. 1999) and Sepsid flies (Puniamoorthy et al. 2010) show similar dynamic patterns of fast evolutionary transitions where the functional role of the spermathecaea has undergone major shifts. Regional environmental differences may have played an initial role in generating this variation by creating spatio-temporal differences in exposure of phenotypic variation to selection thereby potentiating geographic differentiation.

Concluding remarks

Temperature is known to have global effects on ectotherm physiology and much focus has been given to temperature driven phenotypic plasticity and local adaptation in this group of organisms. However, effects of temperature, directly or through pleiotropy, on the development of reproductive morphology and sexual selection dynamics over spatio-temporal scales have received less attention. This is unfortunate because changes in the female reproductive tract may affect the relative fertilization success of alternative male phenotypes. Induced covariation between genitalic and other physiological traits through a common environmental variable is

548 further predicted to generate a reinforcement mechanism facilitating local adaptation. Our study
549 identifies temperature as a potent agent not only enforcing systematic selection on life history and
550 physiological traits, but also systematically moulding and exposing cryptic variation in female
551 reproductive morphology. These conditions set the stage for rapid evolutionary dynamics.
552
553
554

Acknowledgements

We thank Luc Bussiere, Oliver Martin, Barbara Tschirren, Karl Gotthard, Hanne Lovlie, Vincent Debat and one anonymous reviewer for helpful comments on earlier versions of this manuscript. We are also grateful to Eric Postma, Richard Walters and Cait Dmitriew for fruitful discussion regarding data analysis and presentation. DB was supported by a grant from the Swedish Research Council VR, MAS and SSB were supported by grants from the German Research Council DFG, and WUB was supported by a grant from the Swiss National Fund SNF.

References

Allen, A. K. and A. C. Spradling. 2008.The *Sf1*-related nuclear hormone receptor *Hr39* regulates *Drosophila* female reproductive tract development and function. Development 135: 311-321.

Amitin, E. G. and S. Pitnick. 2007. Influence of developmental environment on male- and female- mediated sperm precedence in *Drosophila melanogaster*. J. Evol. Biol. 20: 381-391.

Andrade, C. A. C., Hatadani L. M. and L. B. Klaczko. 2005. Phenotypic plasticity of the aedeagus of *Drosophila mediopunctata*: Effect of temperature. J. Therm. Biol. 30: 518-523.

Angilletta, M. J. 2009. *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press, Oxford.

Arnqvist, G. 1997. The evolution of animal genitalia: distinguishing between hypotheses by single species studies. Biol. J. Linn. Soc. 60: 365-379.

Arnqvist, G. and L. Rowe. 2005. *Sexual Conflict.* Princeton University Press, Princeton, NJ.

Arnqvist, G. and Thornhill, R. 1998. Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition dependence of genital and non-genital morphology in a water strider. Genet. Res. 71: 193-212.

579 Atkinson, D. 1994. *Temperature and organism size – A biological law for ectotherms?* In:
580 Advances in ecological research, Vol. 25 (Begon, M., ed). Academic Press Limited, San Diego.
581 Beese, K., K.Beier and B. Baur. 2006. Coevolution of male and female reproductive traits in a
582 simultaneously hermaphroditic land snail. J. Evol. Biol. 19: 410-418.
583 Beese K., G. F. J. Armbruster, K. Beier and B. Baur. 2009. Evolution of female sperm-storage
584 organs in the Carrefour of stylommatophoran gastropods. J. Zool. Syst. Res. 47: 49-60.
585 Birkhead T.R. 2010. How stupid not to have thought of that: post-copulatory sexual selection. J.
586 Zool. 281: 78-83.
587 Blanckenhorn, W. U. 2009. Causes and consequences of phenotypic plasticity in body size: The
588 case of the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). In: *Phenotypic*
589 *plasticity of insects* : *Mechanisms and consequences* (Whitman D., and Ananthakrishnan T. N.,
590 eds). Science Publishers, Enfield, NH, USA.
591 Blanckenhorn, W. U., Pemberton, A. J., Bussiere L. F., Roembke, J., and Floate K. D. 2010. A
592 review of the natural history and laboratory culture methods for the yellow dung fly, *Scathophaga*
593 *stercoraria*. J. Insect. Sci. 10: article 11.
594 Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens and J. S.
595 White. 2008. Generalized linear mixed models: a practical guide for ecology and evolution.
596 Trends. Ecol. Evol. 24: 127-135.
597 Bussiere, L. F., M. Demont, A. J. Pemberton, M. D. Hall and P. I. Ward. 2010. The assessment of
598 insemination success in yellow dung flies using competitive PCR. Mol. Ecol. Res. 10: 292-303.
599 Civetta, A. and R. S. Singh. 1998. Genetic architecture and evolutionary potential of sexual
600 versus nonsexual traits in the sibling species of the *Drosophila melanogaster* complex. Evolution
601 52: 1080-1092.

602 Clark, A. G., D. J. Begun and T. Prout. 1999. Female x male interactions in Drosophila sperm
603 competition. Science 283: 217-220.
604 Debat, V. and P. David. 2001. Mapping phenotypes: canalization, plasticity and developmental
605 stability. Trends. Ecol. Evol. 16: 555-561.
606 Demont, M., W. U. Blanckenhorn, D. J. Hosken and T. W. J.Garner. 2008. Molecular and
607 quantitative genetic differentiation across Europe in yellow dung flies. J. Evol. Biol. 21: 1492-
608 1503.
609 De Visser, J. A. G. M., J. Hermisson, G. P. Wagner, L. A. Meyers, H. Bagheri-Chaichian, J. L.
610 Blanchard, L. Chao, J. M. Cheverud, S. F. Elena, W. Fontana, G. Gibson, T. F. Hansen, D.
611 Krakauer, R. C. Lewontin, C. Ofria, S. H. Rice, G. von Dassow, A. Wagner and M. C. Whitlock.
612 2003. Evolution and detection of genetic robustness. Evolution 57: 1959-1972.
613 Ding, L. and E. P. M Candido. 2000. Association of several small heat-shock proteins with
614 reproductive tissues in the nematode *Caenorhabditis elegans* Biochem. J. 351: 13–17.
615 Dobzhansky, T. H. and A. M. Holz .1943. A re-examination of the problem of manifold effects of
616 genes in *Drosophila melanogaster*. Genetics 28: 295:303.
617 Dybas , L. K. and H. S. Dybas. 1981. Coadaptation and taxonomic differentiation of sperm and
618 spermathecae in featherwing beetles. Evolution 35: 168-174.
619 Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton
620 Univ. Press, Princeton, New Jersey.
621 Ellegren, H. and Parsch, J. (2007) The evolution of sex-biased genes and sex-biased gene
622 expression. Nature Reviews Genetics 8: 689-698
623 Goldstein, H., W. Browne and J. Rasbash. 2002. Partitioning variation in multilevel models.
624 Understanding statistics 1: 223-231.

625 Hadfield J. 2010. MCMC methods for multi-response generalized linear mixed models: The
626 MCMCglmm R package. Contributed package for R computational and statistical software.
627 Hellriegel, B. and P. I. Ward. 1998. Complex female reproductive tract morphology: Its possible
628 use in postcopulatory female choice. J. Theor. Biol. 190: 179-186.
629 Hochachka, P.W. and G. N. Somero. 2002. *Biochemical adaptation: Mechanism and process in*
630 *physiological evolution*. Oxford University Press, Oxford.
631 Hosken, D. J., T. W. J. Garner and P. I. Ward. 2001. Sexual conflict selects for male and female
632 reproductive characters. Curr. Biol. 11: 489-493.
633 Hosken, D. J., W. U. Blanckenhorn and T. W. J. Garner. 2002. Heteropopulation males have a
634 fertilization advantage during sperm competition in the yellow dung fly. Proc. R. Soc. B. 269:
635 1701-1707.
636 Hosken, D. J. and P. Stockley. 2004. Sexual selection and genital evolution. Trends. Ecol. Evol.
637 19: 87-93.
638 Houle D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of
639 and why it matters. Evolution 45: 630-648.
640 Jennions M. D. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic
641 benefits. Biol. Rev. 75: 21-64.
642 Kamimura, Y. 2007. Possible atavisms of genitalia in two species of earwig (Dermaptera),
643 *Proreus simulans* (Chelisochidae) and *Euborellia plebeja* (Anisolabididae). Arthr. Struc. Dev. 36:
644 361-368.
645 Kondrashov A.S., and F.A. Kondrashov. 1999. Interactions among quantitative traits in the
646 course of sympatric speciation. Nature 400: 351-354.
647 Kraaijeveld K. and F. J. L. Kraaijeveld-Smit and M. E. Maan. 2010. Sexual selection and
648 speciation: the comparative evidence revisited. Biol. Rev. 86: 367-377

649 Kraushaar, U. and W. U. Blanckenhorn. 2002. Population variation in sexual selection and its
650 effects on size allometry in two dung fly species with contrasting sexual size dimorphism.
651 Evolution 56: 307-321.
652 Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity
653 and genetic assimilation. J. Evol. Biol. 22: 1435-1446.
654 Lynch M, Walsh B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates Inc.
655 Sunderland, USA.
656 Mather K. and B. J. Harrison. 1949. The manifold effect of selection. Heredity 3: 131-164.
657 Mayr, E. 1963. *Animal species and evolution*. Harvard University Press, Cambridge.
658 McGuigan, K. and C. M. Sgro. 2009. Evolutionary consequences of cryptic genetic variation.
659 Trends. Ecol. Evol. 24: 305-311.
660 Miller, G. T. and S. Pitnick. 2002. Sperm-female coevolution in *Drosophila*. Science 298: 1230-
661 1233.
662 Miller, G. T. and S. Pitnick. 2003. Functional significance of the seminal receptacle length in
663 *Drosophila melanogaster*. J. Evol. Biol. 16: 114-126.
664 Minder, A. M., D. J. Hosken, P. I. Ward. 2005. Co-evolution of male and female reproductive
665 characters across the *Scathophagidae* (Diptera). J. Evol. Biol. 18: 60-69.
666 Moczek , A. P. 2009. Phenotypic plasticity and the origins of diversity: A case study on horned
667 beetles. In: *Phenotypic plasticity of insects* : *Mechanisms and consequences* (Whitman D., and
668 Ananthakrishnan T. N., eds). Science Publishers, Enfield, NH, USA.
669 Nakagawa S. and H. Schielzeth. 2010. Repeatability for Gaussian and non-gaussian data: a
670 practical guide for biologists. Biol Rev. 85: 935-956.

671 Otronen, M., P. Reguera and P. I. Ward. 1997. Sperm storage in the yellow dung fly *Scathophaga*
672 *stercoraria*: Identifying the sperm of competing males in separate female spermathecae. Ethology
673 103: 844-854.
674 Parker, G. A. and Simmons, L. W. 1994. Evolution of phenotypic optima and copulation duration
675 in dung flies. Nature, 370: 53-56.
676 Phiancharoen, M., C. W. W. Pirk, S. E. Radloff and Hepburn R. 2010. Clinal nature of the
677 frequencies of ovarioles and spermathecae in Cape worker honeybees, *Apis mellifera capensis*.
678 Apidologie 41: 129-134.
679 Pitnick, S., S. T. A. Markov and G. S. Spicer. 1999. Evolution of multiple kinds of female sperm-
680 storage organs in *Drosophila*. Evolution 53: 1804-1822.
681 Pitnick., S., M. Wolfner and S. Suarez. 2009. Ejaculate-female and sperm-female interactions. In:
682 *Sperm Biology: An Evolutionary Perspective* (T. R. Birkhead, D. J. Hosken and S. Pitnick, eds),
683 pp. 247-304. Academic Press, London.
684 Presgraves, D. C., R. H. Baker and G. S. Wilkinson. 1999. Coevolution of sperm and female
685 reproductive tract morphology in stalk-eyed flies. Proc. R. Soc. Lond. B. 266: 1041-1047.
686 Prokupek, A. M., S. I. Eyun, L. Ko, E. N. Moriyama and L. G. Harshman. 2010. Molecular
687 evolutionary analysis of seminal receptacle sperm storage organ genes of *Drosophila*
688 *melanogaster*. J. Evol. Biol. 23: 1386-1398.
689 Prud'homme, B., N. Gompel, A. Rokas, V. A. Kassner, T. M. Williams, S. Yeh, J. R. True and S.
690 B. Carroll. 2006. Repeated morphological evolution through cis-regulatory changes in a
691 pleiotropic gene. Nature 440: 1050-1053.
692 Puniamoorthy, N., M. Kotrba and R. Meier. 2010. Unlocking the "black box": internal female
693 genitalia in *Sepsidae* (Diptera) evolve fast and are species-specific. BMC Evol. Biol. 10: 275
694 Ritchie, M. G. 2007. Sexual selection and speciation. Annu. Rev. Ecol. Evol. Syst. 38: 79-102.

695 Rivera, A. C., J. A. Andres, A. Cordoba-Aguilar and C. Utzeri. 2004. Postmating sexual
696 selection: allopatric evolution of sperm competition mechanisms and genital morphology in
697 calypterigid damselflies (Insecta: Odonata). Evolution 58: 349-359.
698 Rönn, J., M. Katvala and G. Arnqvist. 2007. Coevolution between harmful male genitalia and
699 female resistance in seed beetles. Proc. Natl. Acad. Sci. USA 104: 10921–10925
700 Rutherford S. L. and S. Lindquist. 1998. Hsp90 as a capacitor for morphological evolution.
701 Nature 396: 336-342.
702 Sangster, T. A., S. Lindquist and C. Queitsch. 2004. Under cover: causes, effects and
703 implications of Hsp90-mediated genetic capacitance. Bioessays 26: 348-362.
704 Sauer J. and B. Hausdorf. 2009. Sexual selection is involved in speciation in a land snail radiation
705 on crete. Evolution 63: 2535-2546.
706 Sbilordo S. H., M. A. Schäfer and P. I. Ward. 2009. Sperm release and use at fertilixation by
707 yellow dung fly females (*Scathophaga stercoraria*). Biol. J. Linn Soc. 98: 511-518.
708 Scharf, I., S. S. Bauerfeind, W. U. Blanckenhorn, M. A. Schäfer. 2010. Effects of maternal and
709 offspring environmental conditions on growth, development and diapause in latitudinal yellow
710 dung fly populations. Clim. Res. 43: 115-125.
711 Schlichting, C. D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. Ann.
712 N.Y. Acad. Sci. 1133: 187-203.
713 Schmalhausen, I.I. 1949. *Factors of evolution: The theory of stabilizing selection*. Blakiston,
714 Philadelphia, PA.
715 Shingleton, A. W., C. M. Estep, M. V. Driscoll and I. Dworkin. 2009. Many ways to be small:
716 different environmental regulators of size generate distinct scaling relationships in *Drosophila*
717 *melanogaster*. Proc. R. Soc. B. 276: 2625-2633.

718 Simmons, L. W., G. A. Parker and P. Stockley. 1999. Sperm displacement in the yellow dung fly,
719 *Scatophaga stercoraria*: An investigation of male and female processes. Am. Nat. 153: 302-314.
720 Snook, R.R., L.D. Bacigalupe, and A. J. Moore. 2010. The quantitative genetics and coevolution
721 of male and female reproductive traits. Evolution 64: 1926-1934.
722 Soto, I. M., M. H. Manfrin and E. Hasson. 2008. Host-dependent phenotypic plasticity of
723 aedeagus morphology in a pair of cactophilic sibling *Drosophila* species of the *repleta* group
724 (Diptera, Drosophilidae). J. Zool. Syst. Evol. Res. 46: 368-373.
725 Stearns, S.C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience 39: 436-
726 445.
727 Suzuki, Y. and H. F. Nijhout. 2006. Evolution of a polyphenism by genetic accommodation.
728 Science 311: 650-652.
729 Swanson W.J. and V. D. Vacquier. 2002. The rapid evolution of reproductive proteins. Nat. Rev.
730 Gen. 3: 137-144.
731 Tanaka, K., O. Barmina and A. Kopp. 2009. Distinct developmental mechanisms underlie the
732 evolutionary diversification of *Drosophila* sex combs. Proc. Natl. Acad. Sci. 106: 4764-4769.
733 Teuschl, Y., C. Reim and W. U. Blanckenhorn. 2007. Correlated responses to artificial body size
734 selection in growth, development, phenotypic plasticity and juvenile viability in yellow dung
735 flies. J. Evol. Biol. 20: 87-103.
736 Thüler, K. 2009. *The genetic architecture of female reproductive morphology and its influence on*
737 *male and female life history in yellow dung flies Scathophaga stercoraria (L.)* Doctoral thesis,
738 Zoological Museum, University of Zurich.
739 Thüler, K., L. F. Bussiere, E. Postma, P. I. Ward and W. U. Blanckenhorn. 2011. Genetic and
740 environmental sources of covariance among internal reproductive traits in the yellow dung fly. J.
741 Evol. Biol. *doi: 10.1111/j.1420-9101.2011.02280.x.*

742 Tregenza, T., F. Attia, and S. S. Buahaiba. 2009. Repeatability and heritability of sperm
743 competition outcomes in males and females of *Tribolium castaneum*. Behav. Ecol. Sociobiol.
744 63:817–823.
745 Waddington, C. H. 1953. Genetic assimilation of an acquired character. Evolution 7: 118-126.
746 Van Dyken, J. D. and M. J. Wade. 2010. The genetic signature of conditional expression.
747 Genetics 184: 557-570.
748 Ward, P. I. 2000. Female choice in the yellow dung fly *Scathophaga stercoraria* (L.). Evolution
749 54: 1680-1686.
750 Ward, P. I., A. J. Wilson and C. Reim. 2008. A cost of female choice in the yellow dung fly.
751 Genetica 134: 63.67.
752 West-Eberhard, M. 2003. *Developmental Plasticity and Evolution*. Oxford University press, New
753 York.
754 Wexelsen, H. 1928. Two new mutant characters on the spermathecae of the females of
755 *Drosophila melanogaster*, cell-degeneration and supernumerary spermathecae. Genetics 13: 389-
756 400.
757 Whitlock, M. C. 2008. Evolutionary inference from Qst. Mol. Ecol. 17: 1885-1896.
758 Yeates, D. K. 2002. Relationships of extant lower Brachycera (Diptera): a quantitative synthesis
759 of morphological characters. Zool. Scripta 31: 105-121.
760

Fig 1

Above: 3s-phenotype. Below: 4s phenotype with the three arrows indicating that the left pair of spermathecae share the same entrance to the bursa copulatrix through a joining of the spermathecal ducts (indicated by a fourth arrow). Other intermediate forms between the 3s and 4s phenotypes exist. On rare occasions two spermathecal compartments can be joined on one side and thus might effectively function as a single compartment. In this study however, we scored all intermediate forms as 4s-phenotypes.

Fig 2

The sampling sites of the natural populations used in the common garden in 2007 and 2009 designated by open circles. The numbers within (or next to) circles reports the number of families started in the common garden for respective population. Closed circles show the sampling sites for the populations scored for Fst in Demont et al. (2008).

Fig 3

Parallel responses of mean 4s-phenotype expression (closed circles) and growth rate (open circles) to rearing temperature across all populations. Means ± confidence limits, which, for growth rate are smaller than the circles.

Fig 4

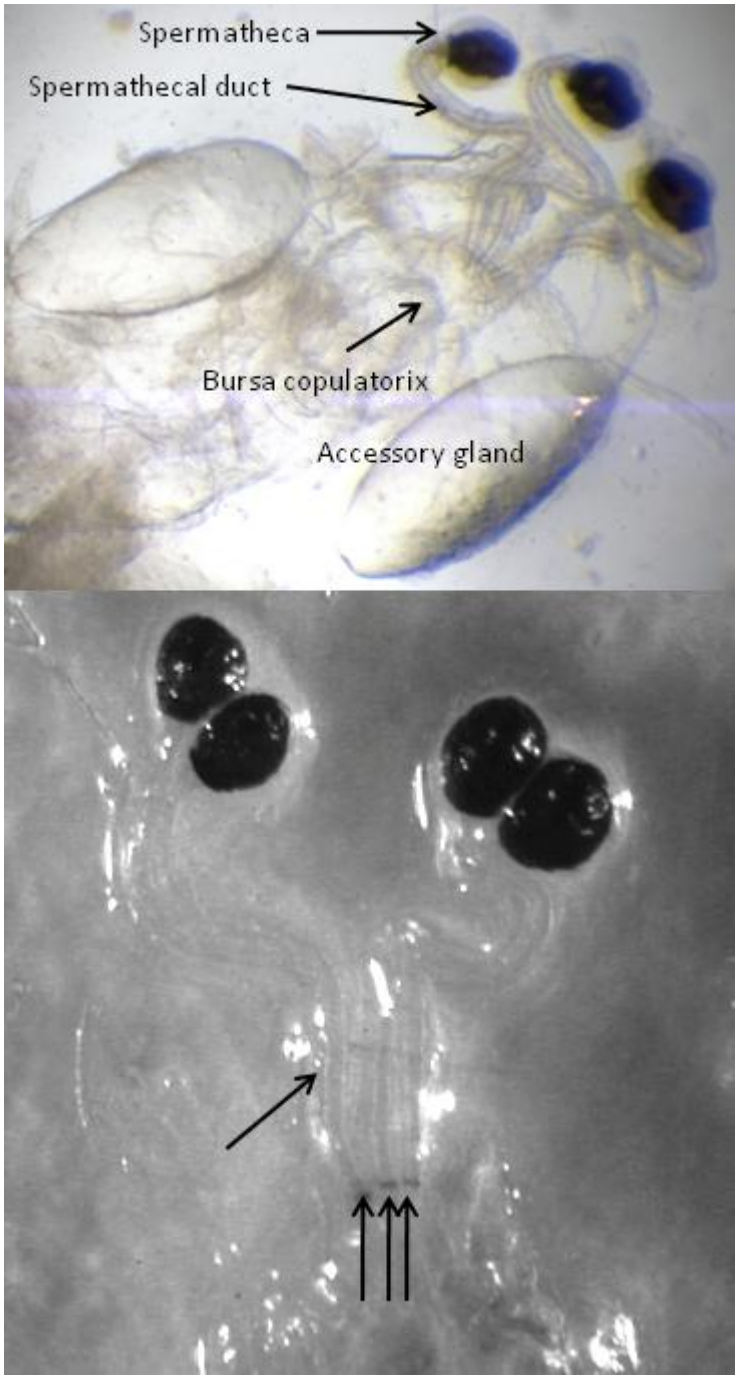
Total phenotypic (open circles) and genetic (closed circles) variance for the spermatheca polymorphism in the different growth conditions. Total phenotypic variance (V_P) for a binomial trait is equal to the product of the frequencies of the two possible outcomes: p*(1-p). Total genetic variance (V_G) is equal to the product of the phenotypic variance and the broad sense

heritability, estimated as twice the intraclass correlation for family in our sample using a full-sib design. Confidence limits for V_G were obtained using the estimates from the Bayesian analyses.

Fig 5

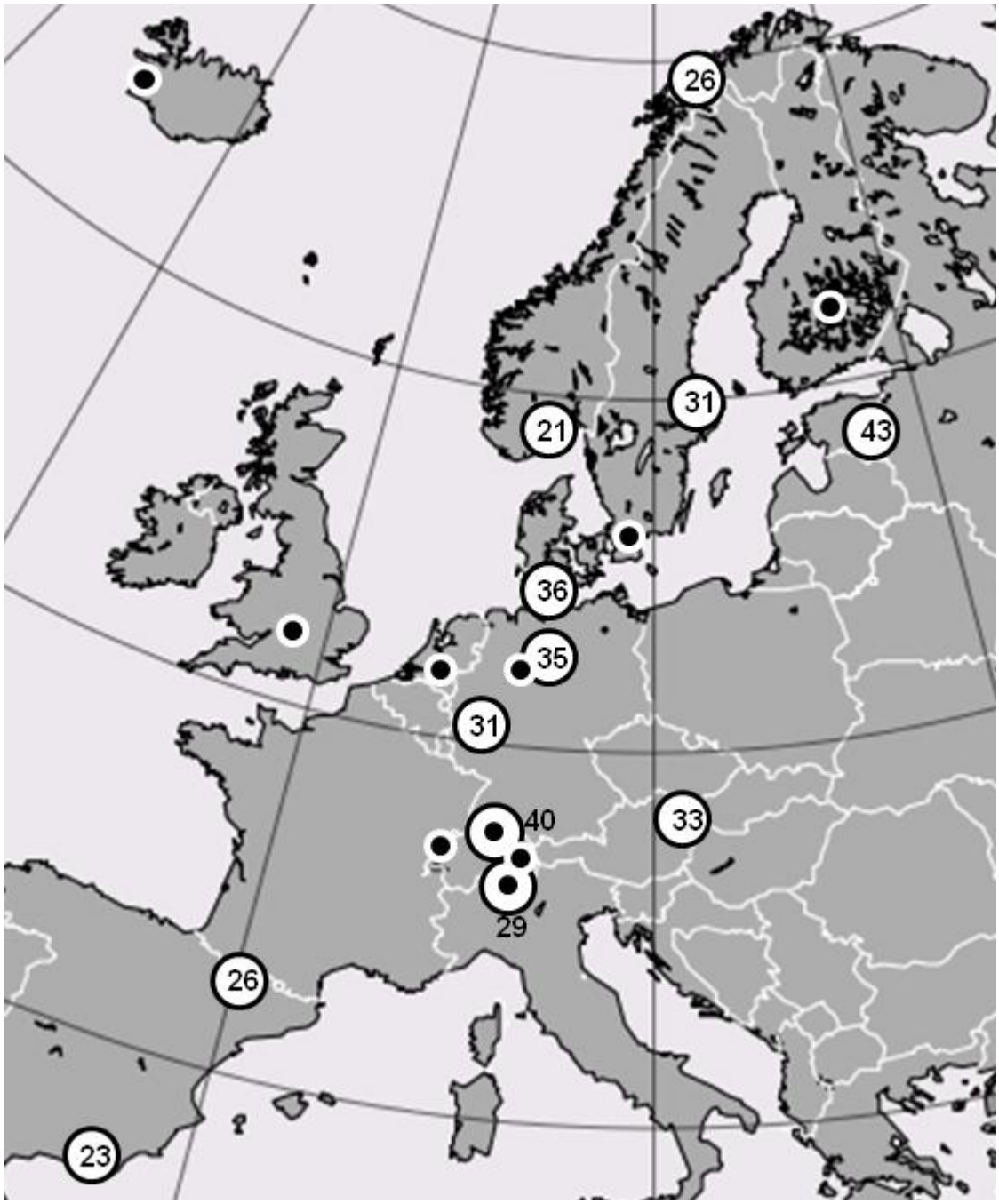
Parallel latitudinal clines for 4s-phenotype frequency (closed circles) and growth rate (open circles) based on population means for females raised at 12°C.

792 Fig 1


Spermatheca
Spermathecal duct
Bursa copulatorix
Accessory gland

793

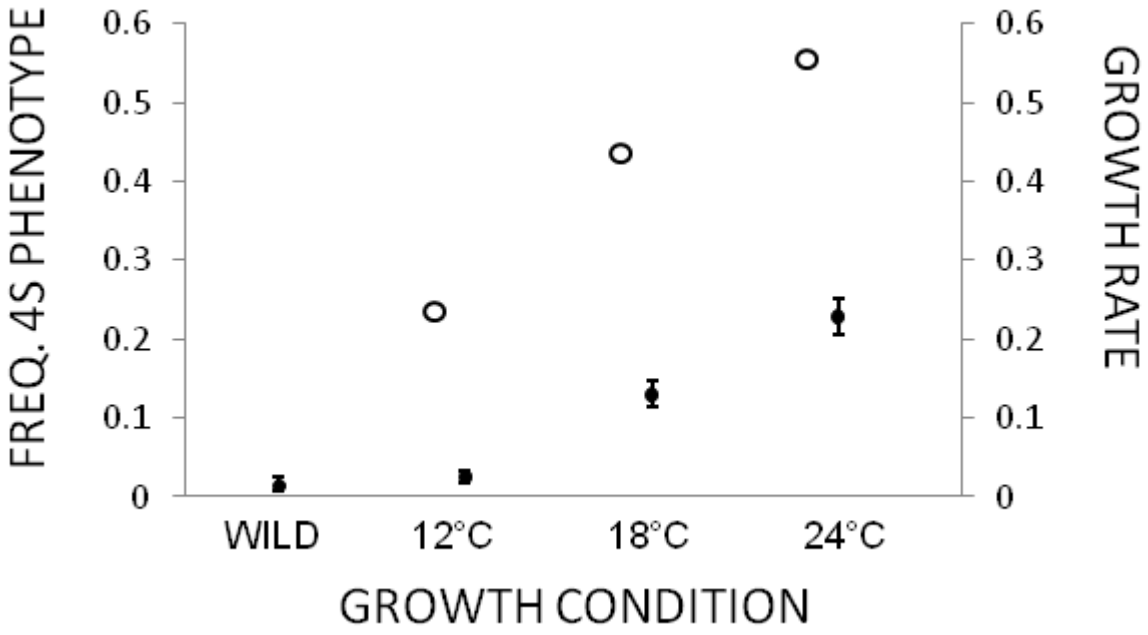
794 **Fig 2**



795

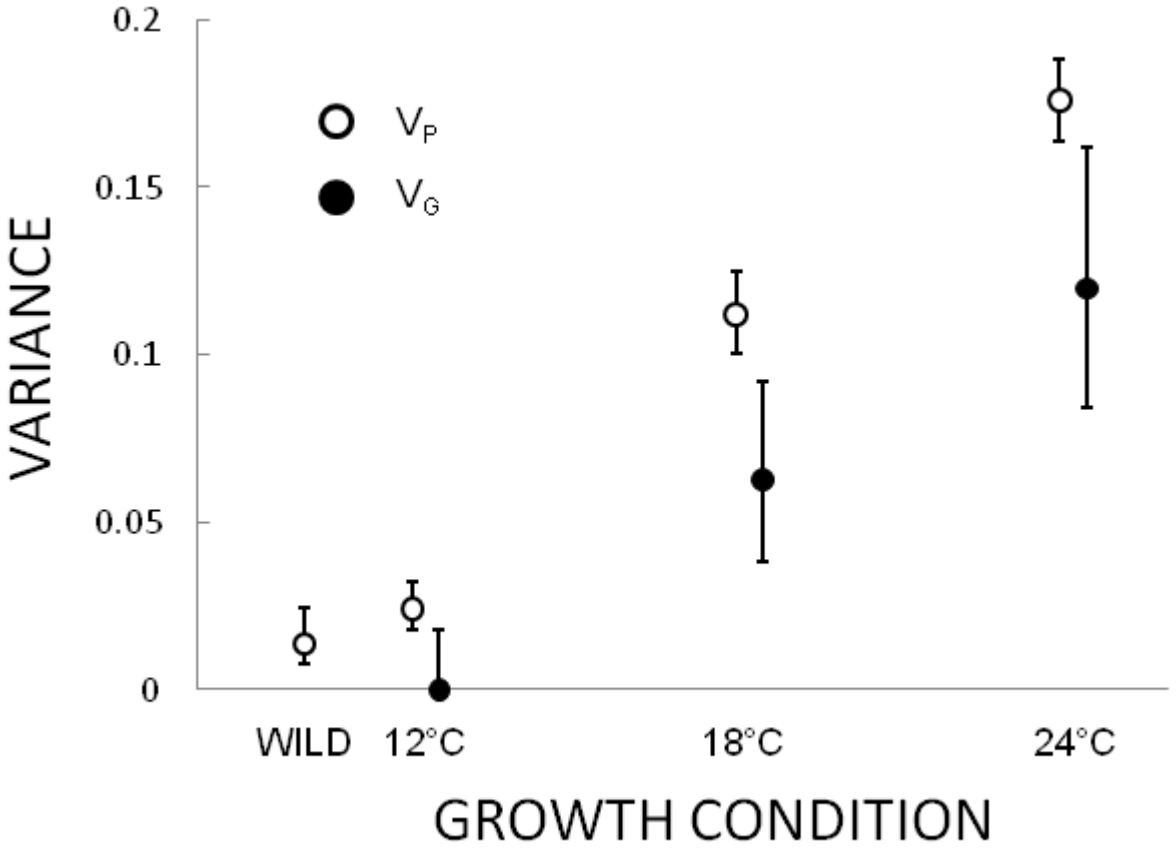
796

797 **Fig 3**


FREQ. 4S PHENOTYPE
GROWTH RATE
0.6
0.5
0.4
0.3
0.2
0.1
0
WILD
12°C
18°C
24°C
GROWTH CONDITION

798

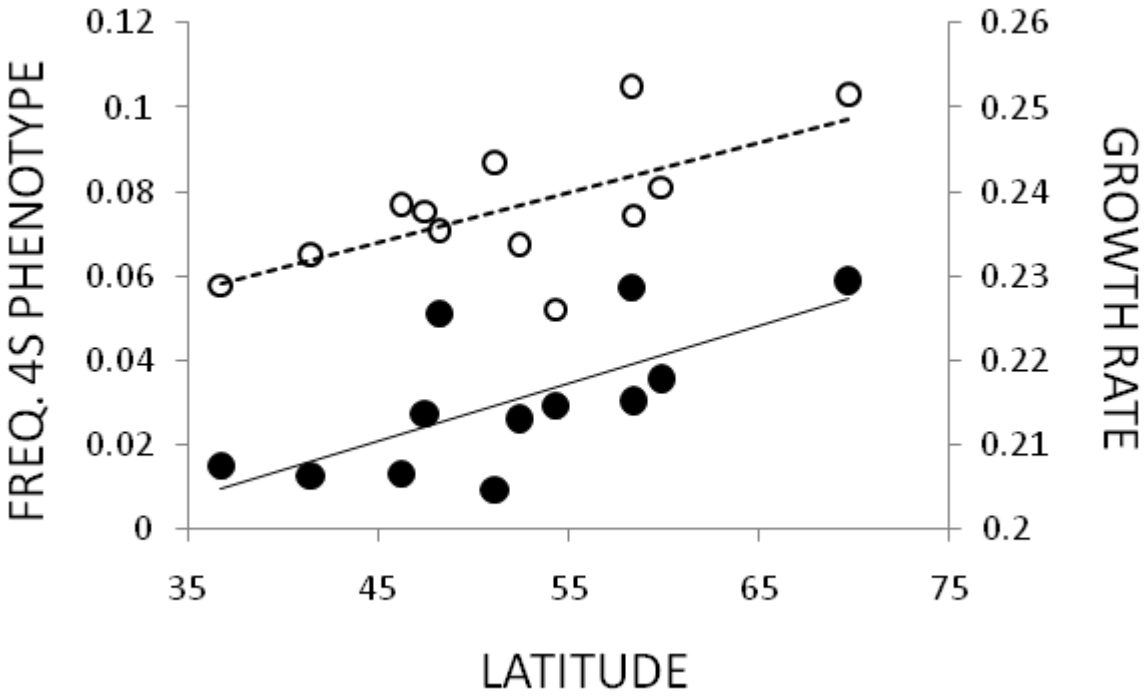
799 **Fig 4**



800

801

802 **Fig 5**


0.12
0.1
0.08
0.06
0.04
0.02
0
FREQ. 4S PHENOTYPE
0.26
0.25
0.24
0.23
0.22
0.21
0.2
GROWTH RATE
35
45
55
65
75
LATITUDE

803

804 **Table 1:** Qst, and intraclass correlations for population effects and family effects calculated
805 within population, for spermathecal number and life history traits. Modes and 95% credible
806 intervals (CI) were estimated with Bayesian statistics. For such non-parametric statistics the
807 mode, giving the most common value (with highest posterior density) in the simulation is a better
808 estimate than the mean. REML statistics for the effects obtained from the mixed modeling
809 analyses conducted on each temperature treatment separately are presented in the three rightmost
810 columns; The Chi-2 value represents the Wald-statistic for the likelihood-ratio of the two
811 compared models (with and without the specific effect of interest), and d.f. gives the number of
812 additional degrees of freedom used up by the more complicated model (incorporating the effect).
813 Both analysis methods included the same number of females reported in the "sample"- columns.
814 Note that the calculated Qst for spermathecal number in 12°C is uninformative since family
815 effects could not be estimated with precision due to low expression of the 4s-phenotype. We
816 conclude i) that life history traits show signs of local adaptation whereas spermathecal number in
817 18 and 24°C seem to evolve neutrally, and ii) life history traits show higher heritability and
818 genetic variance than spermathecal number but that warm temperature reveals ample hidden
819 genetic variance for the spermathecal polymorphism. The family effects for all traits are likely
820 overestimated due to common environment effects and possible maternal effects. This in turn is
821 likely to produce underestimated Qst:s. However, this possibility is not likely to have influenced
822 the conclusions from our analyses. First of all, Qst:s for life history traits are likely downward
823 biased, whereas the estimated Qst:s for spermathecal number remain largely unaffected by
824 reasonable changes of family effects. This is because the population effects for spermathecal
825 number were estimated to be zero producing Qst:s of zero regardless. Secondly, our genetic
826 correlations for growth rate and spermathecal number between 18 and 24°C, excluding common
827 rearing environment effects, show high values (G_{cov} = 0.30 and 0.31 respectively), supporting

828 strong genetic regulation as suggested by analyses within each of these temperatures. In addition,
829 previous analyses using artificial selection on both spermathecal number (Ward 2000, Ward et al.
830 2008) and body size (Teuschl et al. 2007) indicate that genetic components for growth rate and
831 the spermatheca polymorphism are substantial.
832
833

spermathecal number

temperature	component	sample	mode	Low.CI	Up.CI	Chi-2	d.f.	p-value
12°C	**fam**	1726	0.00	0.00	0.37	6.66	1	**0.010**
18°C	**fam**	1686	0.28	0.17	0.41	24.0	1	**<0.001**
24°C	**fam**	1413	0.34	0.24	0.46	46.3	1	**<0.001**
12°C	**pop**	1726	0.00	0.00	0.11	3.55	11	0.98
18°C	**pop**	1686	0.00	0.00	0.10	17.9	11	0.083
24°C	**pop**	1413	0.00	0.00	0.08	16.0	11	0.14
12°C	**Qst**	1726	0.00	0.00	0.75			
18°C	**Qst**	1686	0.00	0.00	0.09			
24°C	**Qst**	1413	0.00	0.00	0.06			

growth rate

temperature	component	sample	mode	Low.CI	Up.CI	Chi-2	d.f.	p-value
12°C	**fam**	530	0.40	0.33	0.49	88.0	1	**<0.001**
18°C	**fam**	1227	0.32	0.26	0.37	145.8	1	**<0.001**
24°C	**fam**	1057	0.29	0.23	0.35	108.4	1	**<0.001**
12°C	**pop**	530	0.08	0.02	0.34	66.1	11	**<0.001**
18°C	**pop**	1227	0.08	0.02	0.23	47.6	11	**<0.001**
24°C	**pop**	1057	0.04	0.01	0.14	33.6	11	**<0.001**
12°C	**Qst**	530	0.07	0.01	0.25			
18°C	**Qst**	1227	0.06	0.01	0.19			
24°C	**Qst**	1057	0.04	0.00	0.13			

development time

temperature	component	sample	mode	Low.CI	Up.CI	Chi-2	d.f.	p-value
12°C	**fam**	1219	0.52	0.46	0.58	373.2	1	**<0.001**
18°C	**fam**	1757	0.44	0.39	0.49	446.6	1	**<0.001**
24°C	**fam**	1500	0.54	0.49	0.60	444.9	1	**<0.001**
12°C	**pop**	1219	0.19	0.09	0.48	75.3	11	**< 0.001**
18°C	**pop**	1757	0.03	0.00	0.14	30.5	11	**0.001**
24°C	**pop**	1500	0.02	0.00	0.10	24.5	11	**0.011**
12°C	**Qst**	1219	0.11	0.04	0.30			
18°C	**Qst**	1757	0.02	0.00	0.08			
24°C	**Qst**	1500	0.01	0.00	0.05			

body size

temperature	component	sample	mode	Low.CI	Up.CI	Chi-2	d.f.	p-value
12°C	**fam**	1097	0.40	0.32	0.47	71.20	1	**< 0.001**
18°C	**fam**	1241	0.27	0.21	0.33	99.0	1	**< 0.001**
24°C	**fam**	1068	0.18	0.11	0.23	38.6	1	**< 0.001**
12°C	**pop**	1097	0.05	0.01	0.16	20.60	11	**0.037**
18°C	**pop**	1241	0.05	0.02	0.18	45.0	11	**< 0.001**
24°C	**pop**	1068	0.03	0.01	0.10	27.9	11	**0.003**
12°C	**Qst**	1097	0.03	0.01	0.11			
18°C	**Qst**	1241	0.04	0.01	0.18			
24°C	**Qst**	1068	0.04	0.01	0.15			